EFFICACY OF NEWER DISINFECTANTS ON CANDIDA ALBICANS INFECTED SILICONE BASED DENTURE SOFT LINER

Dissertation submitted to

THE TAMILNADU Dr.M.G.R.MEDICAL UNIVERSITY

In partial fulfillment for Degree of

MASTER OF DENTAL SURGERY

BRANCH VI – PROSTHETIC DENTISTRY INCLUDING CROWN AND BRIDGE AND IMPLANTOLOGY

APRIL 2011

RAJAS DENTAL COLLEGE

Raja Nagar, Kavalkinaru – 627105, Tirunelveli District

DCI Recognition No. DE-3 (44)-93/2246, Dated 09/11/1993
Affiliated to the Tamil Nadu Dr. M.G.R. Medical University, Chennai

DEPARTMENT OF PROSTHETIC DENTISTRY

INCLUDING CROWN AND BRIDGE AND IMPLANTOLOGY

CERTIFICATE

This is to certify that this dissertation entitled **" EFFICACY OF NEWER DISINFECTANTS ON CANDIDA ALBICANS INFECTED SILICONE BASED DENTURE SOFT LINER"** is a genuine work done by ***Dr. Vinni Mary Oommen*** under my guidance during her post graduate study period between 2008-2011.

This Dissertation is submitted to THE TAMILNADU Dr. M.G.R MEDICAL UNIVERSTY, in partial fulfillment for the degree of **MASTER OF DENTAL SURGERY IN PROSTHETIC DENTISTRY INCLUDING CROWN AND BRIDGE AND IMPLANTOLOGY - BRANCH VI.** It has not been submitted (partial or full) for the award of any other degree or diploma.

Date:

Place :

Dr.ArunKumarG, M.D.S

Head Of The Department

Department OfProsthetic Dentistry

Including Crown And Bridge And Implantology

Rajas Dental College AndHospital

Kavalkinaru.

Dedicated To

My Parents And Brother

Acknowledgement

First of all I reveal my deep sense of gratitude to the Lord Almighty for helping me overcome all odds and for being the unfailing source of support, comfort and strength throughout the completion of the study.

I am sincerely grateful to ***Dr. Arun Kumar G,*** *Professor and Head of the Department for his constant encouragement, help, guidance, advice and care throughout the course of this study and my entire P.G Curriculum.*

I wish to thank my Principal ***Dr. Suresh Satyashekar*** *for his support.*

I also express my deep sense of gratitude and my sincere thanks to ***Dr. Sunitha, Dr.Dadu George, Dr. Paulin Vijay Chandran, Dr. Appukannu , Dr. Aarthi, Dr.Somasundaram, Dr. Lin, Dr. Giri*** *for their encouragement, help and advice throughout the study.*

I am extremely grateful to ***Dr. PunnenKurien, Mr. Manuel Thomas, Mr. Iype, Mr.Abin Varghese*** *of TIES, Kottayam who guided me throughout the tenure of my study.*

I thank my colleagues ***Dr. Ramesh, Dr. Mahesh, Dr. Josna, Dr. Joephin, Dr.Madhu*** *for their help and constant support.*

I thank our department staffs for their timely assistance and help throughout the study.

My sincere thanks to all those who have helped me directly and indirectly.

My heartfelt gratitude to ***my parents and friends*** *for their affection and support throughout my course of the study.*

ABSTRACT

Title: Efficacy of newer disinfectants on *Candida albicans* infected denture soft liners

Mesh Words: Newer disinfectants, soft denture liners, *Candidaalbicans*

Purpose: The purpose of the study was to find newer disinfectant solutions which were less cytotoxic and more nature based for the removal of *Candida albicans*from infected denture soft liners.

Aim: The aim of the study was to evaluate the effectiveness of three new disinfectants- Superoxidised solution, Methanolic extracts of *Zingiberwightianum, Zingiberofficinale* in reducing the colony forming units of *Candida albicans* adhered to silicone based denture soft liners.

Materials and methods: Three disinfectant solutions (Superoxidised water, extracts of *Zingiberofficinale* and *Zingiberwightianum*) were used in this study to test the effectiveness in disinfecting *Candida albicans* infected long term denture soft liners (Mucopren Soft, Kettenbach GmbH & Co.KG, Germany). The liner specimens (2cm x1cm x2mm) were subjected to disinfection treatments with the 3 solutions under study for different time exposures of 1 minute, 5 minutes, 30 minutes. The zones of inhibition were also checked for the *Zingiber* extracts to check their effectiveness.

Result: The *Zingiberofficinale* (Ginger) extract showed the maximum efficacy among the three solutions. It showed complete disinfection of the infected soft liners at both 5minutes and 30 minutes of exposure. *Zingiberwightianum*(Hill Ginger) completely eliminated *Candida albicans* from the soft liners at 30 minutes of exposure. Superoxidised water showed the maximum efficacy at the least time of exposure.

Conclusion: Extract of *Zingiberofficinale* showed the maximum effectiveness of all the three disinfectants in this study, followed by extract of *Zingiberwightianum*. The effectiveness of Superoxidised water was seen to be reducing with increased time of exposures.

CONTENTS

LIST OF FIGURES AND TABLES

TABLES

TABLES

INTRODUCTION

Denture soft liners provide a cushion between the hard denture base and supporting tissue and are used to relieve the pressures transmitted by the dentures on the oral mucosa. The soft liners are mostly used as a therapeutic measure for patients who cannot tolerate the stresses induced by dentures. The liner permits wider distribution of forces and absorption of impact forces that are involved in functional and parafunctional movements. The most commonly used soft liners are plasticized acrylics and silicone rubber. They have been found to provide very good patient satisfaction on the basis of comfort and has been widely used globally. One of the major drawback they face is that they carry a risk of supporting yeast growth leading to oral fungal infections[1,3,21,25].

The prolonged usage of soft liners results in microbial colonization which include many varieties of bacteria, fungi etc. Many species of *Candida* are found housing within the microbial flora on the denture soft liner. The debris that accumulates in the pores of resilient liners provide nutrients to support mycotic growth.

Candidiasis is found to be the most common type of oral fungal infection which can be associated with the formation of biofilms on prosthetic

surfaces[14] . The main causative organism is revealed to be *Candidaalbicans*which paves way for Oral candidasis, denture stomatitis, angular cheilitis etc[1,9]. Many disinfectants are available for the control of dental liner related infections. Numerous studies on highly effective disinfectants like chlorhexidine, glutaraldehyde, sodium hypochlorite, iodophors have shown that they exhibit cytotoxic effects in patients using them.[2,4,8] Due to their cytotoxicity, the need to search for alternative solutions which are more herbal or natural in origin remained. Studies in the past has shown that Superoxidised solution and the Ginger rhizome has been quite effective against *Candida albicans*.[10,12,32,35,43] Hence in this study, an attempt is made to see the efficacy of such alternative solutions with antifungal potential in removing *Candida albicans* from infected long term denture soft liners as it has not been tried out earlier.

Herbal formulations are significant and the antimicrobial activity of several medicinal plants are investigated thoroughly in recent decades. The present study is an attempt to look at the efficacy of *Zingiberwightianum, Zingiberofficinale* and Superoxidised water in the removal of *Candida albicans* adhered to silicone based soft liners. The study also focuses to compare the antimicrobial activity at different time intervals [1minute, 5minutes, 30minutes]. It is expected that this study would generate a baseline

data with regard to anticandidal activity and removal of *Candida albicans* from denture soft liners with nature based disinfectants.

AIMS AND OBJECTIVES

The aim of this study was:

1. To evaluate the effectiveness of three new disinfectants – Superoxidised solution, Methanolic extracts of *Zingiberofficinale* (Ginger), *Zingiberwightianum* (Hill Ginger) in reducing the colony forming units of *Candidaalbicans* adhered to silicone based denture soft liners.
2. To determine the effectiveness of these solutions in varying time of exposures.

REVIEW OF LITERATURE

Mahmoud Khamis et al (1980)[1] compared the effect of two types of tissue conditioning materials, alone or in combination with antiseptics or antibiotics on the oral bacteriologic status of complete denture wearers. The bacteriologic changes as well as the clinical results were related to the length of time that dentures were worn. It was seen that when using an antibiotic or antiseptic for long duration, the oral bacterial flora are replaced by other pathogenic micro organisms especially gut derived gram negative bacilli.

LasseAnsgarSkoglund et al (1982)[2] reported 3 severe cases of desquamative mucosal reactions due to Chlorhexidinegluconate mouthwash. These observations were studied and the possible alternative to the use of 0.1% Chlorhexidinegluconate as mouthwash is suggested.

P.S Wright et al (1985)[3] conducted a study which included 53 persons wearing soft lined dentures. Yeasts were isolated from the soft liners on the dentures. It was seen in this study that the increased isolation of yeasts on the fitting surface of the soft lined mandibular dentures was not associated with an increased incidence of inflammatory changes in the mandibular denture bearing mucosa. This study was unable to demonstrate a relationship

between the presence of yeasts on the soft lining materials and the condition of the surface of the soft liner. Also there was no significant relationship between the isolation of yeasts and the clinical appearance of the mandibular denture bearing mucosa.

Jane McCourtie et al (1985)[4] conducted an invitro study and assessed the difference in adherence of *Candida albicans* to denture acrylic pretreated with ChlorhexidineGluconate. It was seen that maximum inhibition was achieved for 2% chlorhexidine incubated for 30 minutes at room temperature. Inhibition of adherence was greatest when the organisms were grown in conditions that enhanced adherence the most. Yeast grown in high concentration of galactose, which were the most adherent to acrylic, were also the most sensitive to fungicidal action of ChlorhexidineGluconate, whereas those grown in a low concentration of glucose were the least adherent and also the most resistant.

Sharon Hill et al(1991)[5] in their study determined the invitro antimicrobial an cytotoxic concentrations of glutaraldehyde and formocresol. Cytotoxicity was evaluated on tissue cultures of pulp fibroblasts and HeLacela at minimal cidal concentrations and at 10 and 100 fold dilutions. Cells directly exposed to glutaraldehyde were seen to retain their normal

shape and tissue pattern, whereas cells indirectly exposed to vapours were seen to continue proliferation. In this study both were shown to exhibit cytotoxic effects with glutaraldehyde showing a little lesser compared to glutaraldehyde.

C scully (1994)[6] described a detailed current knowledge on *Candida* and Oral candidiosis together with new therapeutic regimens employed in treating theses mycoses. *Candida albicans* is said to be an opportunistic pathogen and is found to be the predominant organism of most candidiosis. It is mostly seen in the immunocompromised persons. Due to increased cases of HIV and resistance to antifungals, Candidasis is on the rise. Diagnosis is done by physical examination and by smear test. Secretion of antimicrobial proteins and peptides is decreased in saliva of patients with oral candidiosis.

P.S Wright(1994)[7] in his article describes a study on 22 patients provided with Molloplast B soft lining materials in their mandibular complete dentures over a period of 9 years. The condition of Molloplast lining was compared with an unused processed soft lining material for physical integrity, surface detail, adhesion to the denture base, colour and odour. Few of the patients experienced pain and soreness under the mandibular denture inspite of the soft lining. Many others showed deteriorative changes which ultimately

led to replacement. In many cases the soft liner has shown to outlast the acrylic teeth.

Babich H et al(1995)[8] conducted a study to assess the potency of chlorhexidine on the length of exposure and the composition of the exposure medium, It was found to be dependent. The adverse effects of chlorhexidine on the plasma membrane were suggested by the leakage of lactic acid dehydrogenase from chlorhexidine treated cells by the increased permeability of chlorhexidine treated liposomes to Ca^{2+} . The toxicity was seen to be progressively lessened as the content of fetal bovine serum in the exposure medium was increased.

M.G.J Waters et al (1997)[9] in their journal has compared the extent of candidal adherence of two experimental soft lining materials with a commercially available soft lining material and an acrylic resin denture base and they concluded that that adherence of *Candida albicans*to the two experimental silicone soft lining materials was significantly less than that for an acrylic resin denture base and a commercially available soft lining material

Tae Youn Chi et al (1998)[10] in their study tested Medilox against 25 strains of bacteria and two strains of fungi for various periods. All strains of bacteria and fungi were seen to be killed within 30 seconds. This study

showed that the superoxidised water disinfectant was effective for the disinfection of commonly isolated bacteria and yeast from hospital, but less effective against spore forming bacteria.

Pizzo G et al (1998)[11] published a study which investigated the invitro antifungal activity of five commercially available mouth rinses containing chlorhexidine. The minimum fungicidal concentration against yeast and the kill times were observed and they showed a significant difference in values. They concluded that the chlorhexidine containing mouth rinses maybe used as an alternative to conventional antifungal drugs in the management of oral candidiasis.

Selkon et al (1999)[12] conducted a study on a new super oxidized water Sterilox. It was tested against *M.tuberculosis, M.aviumintracellulare, M.chelonae, E.coli, E.faecalis, Pseudomonas aeruginosa, Bacillus subtilis,* methicillin resistant *S.aureus, Candida albicans,*polio type 2, HIV . Under clean conditions, freshly generated Sterilox was found to be highly effective against these microorganisms and it was seen that a greater reduction of micro organisms was seen in 2 minutes or less.

Shetty N et al (1999)[13] published a study where Sterilox was studied as a new superoxidised water , which contains a mixture of oxidizing

substances. This was tested against several micro organisms including *Candida* using membrane filters. Maximum effectiveness was seen to be in 2 minutes. Sterilox was suggested as an alternative in endoscopy units as a potent microbicidal agent. Manufacture claims it to be non corrosive to metal and non toxic to biologic tissues.

Susan L. Zunt (2000)[14] published an article that reviews about the common clinical types of oral candidasis, its diagnosis and current treatment modalities with emphasis on the role of prevention of recurrence in the susceptible dental patient. The development of oral candidasis is opportunistic, often due to changes in local or systemic factors. Multiple strategies emphasizing excellent oral hygiene with disinfection of removable prosthesis will often be required in the patients susceptible to recurrences of Candidasis.

Middleton AM et al (2000)[15] in their study compared superoxidised water and glutaraldehyde for the disinfection of contaminated bronchoscopes. It showed that super oxidized water showed maximum efficiency at a lesser time than glutaraldehyde. It was concluded that the superoxidised solution is an effective alternative mycobacterial agent to the established disinfectants for

the disinfection of bronchoscopes due to its non toxic nature and the reduction in the viable counts demonstrated in this study.

Kishijiro et al (2000)[16] investigated the application of superoxidised water on resilient denture liners for plaque control. The electrolysed water was seen to change the rubber hardness of the liner material whereas the surface roughness and the weight of these liners were not changed. It was concluded that only few effects take place on property of resilient liners and hence it can be applied for the cleaning of soft liners.

Richard D. Cannon et al (2001)[17] published an article that has focused on the importance of colonization in manifestation of oral candidiasis. Host defences act to remove the invading yeast and so the immune system defects are the major risk factors for oral candidasis. *Candida albicans* possesses several features that enable it to evade or overcome host defences and colonise the mouth. The prevention of Candidal colonization may be prevented by immunizing the host or by physical interference with adherence mechanisms.

William A. Rutala et al (2001)[18] has compared the new methods of disinfection which included Surfacine, orthophthaldehyde, Super oxidised water and new methods of sterilization. The super oxidized water has been

shown to be non toxic to biological tissues. The antimicrobial activity of Superoxidized water has been tested against various micro organism including *Candida albicans*. They have been found to be very effective but as seen in other studies their effectiveness reduces in presence of organic matter. Superoxidised water had disadvantages of having the need of expensive equipment for its production and also limited use life.

Yuki Nagamatsu et al (2001)[19] in their journal focuses on the effectiveness of electrolysed water on bacteria. It was seen to be completely eliminating the bacteria on the denture base in an exposure of 1 minute. Both the strong and weak electrolysed acid waters were found very effective and hence they were applicable to the disinfectant for acrylic denture base showing bactericidal activities in significantly shorter treatments.

A Akpan, R. Morgan (2002)[20] describes in detail the classification of Oral candidiasis, the risk factors involved, the management and prognosis. Candidasis is an opportunistic infection of the oral cavity caused by an overgrowth of the *Candida* species. The incidence depends on age and certain predisposing factors. Risk factors included impaired salivary gland functions, drugs, dentures, high carbohydrate diet, smoking, diabetes mellitus,

malignancies, immunosuppressive conditions. Management involves history taking, examination and appropriate antifungal treatment.

Mary E. Brosky et al (2003)[21] in their study shows a purpose to count and speciate*Candida* isolated from two resilient denture liners, Molloplast B and MPDS-SL. A group of 20 patients was included in the study. It was seen that there was no significant difference in the growth of *Candida* on Molloplast B and MPDS-SL. The rates of culture positive testing did not differ between the two resilient denture liners.

M. Urata et al(2003)[22] tested the microbicidal activity of superoxidised water ,ozonated water, 0.05% chlorhexidine and 2% glutaraldehyde were against seven strains of clinical micro organism isolates in this study. This study supports that the bactericidal effects of electrolysed water and ozonated water decreases in the presence of organic matter. They have concluded that super oxidized water have powerful microbicidal activities and can be used safely.

C.Ficker et al (2003)[23] examined the constituents of *Zingiberofficinale* which shows the anti fungal effects in this study. Gingerols and Gingerdiol were the main antifungal principle constituents. These compounds were found to be active against 13 human pathogens. It was

concluded that the identified compounds would assure the antifungal property of ginger.

Renata MC Rodrigues Garcia et al (2003)[24] evaluated the effects of a denture cleanser on weight change, roughness, tensile bond strength on 2 denture resilient lining materials. Results showed that specimens immersed in Polident showed higher weight changes than those immersed in water. Significant difference in roughness was seen between treatments. Hence it was concluded that specimens immersed in Polident demonstrated increased weight changes of resilient liners when compared with tap water, but surface roughness and tensile bond strength were unaffected.

Bulad K et al (2004)[25] in their study monitors the interaction of *Candida albicans* on denture soft lining materials. They have compared the short term adhesion of *Candida albicans* to six denture lining materials and to monitor any long term penetration of material by the yeast. In this study none of the materials produced a zone of inhibition when compared with the nystatin control. It was seen that the rougher surfaces showed more number of colonies than on the smooth surfaces.

Landa Solis C et al (2005)[26]evaluated the invitro antimicrobial and antiviral activities of this new superoxidised water against few organisms like

Staphylococcus aureus, E.coli, Pseudomonas aeruginosa, Salmonella typhi ,and *Candida albicans* in pure culture. The results showed maximum effectiveness within 2-3 minutes of exposure to the superoxidised water. It was concluded that this superoxidised water exerted a wide antimicrobial spectrum with major advantages over acidic superoxidised water.

McCullough et al (2005)[27] in this article reviews the current concepts of mycology and candidal infections as they relate to discussed. Proposed classification for the presentation of oral candidiosis is outlined. The diagnosis and the principles of management are discussed. The therapeutic agents available for the management of these infections are presented and a treatment protocol for the management of patients with oral candidiosis is given.

Douglas G. Benting et al(2005)[28]conducted a study where six resilient denture liners were exposed to two effervescent denture cleansers to evaluate change in compliance over a simulated 1 year time interval. It was concluded that the exposure of the liners to the denture cleansers resulted in an increase in flexibility. It was shown to be dependent on the type of cleanser and time. Chairside materials seemed to change more than laboratory processed liners.

K. Rideout et al (2005)[29] in this journal has compared the cytotoxicity of glutaraldehyde with its alternatives. This study assesses the current practices regarding the use of high level disinfectants and predicts the relative toxicities of each product. Glutaraldehyde was reported as being toxic and having health effects for the employees using this product. The potential risks of all high level disinfectants are serious and hence regulators and users are faced with important risk management decisions before and after they have been introduced to their workplaces.

Andre A Gutierrez (2006)[30]tested the bactericidal and fungicidal activity of superoxidisedwater against a variety of species in vitro. Maximum effectiveness against *Candida albicans* was seen within an exposure time of 30 seconds. Toxicology studies showed that microcyn technology does not cause toxicity, irritation, sensitivity. This was claimed to be the first stable and superoxidised solution. The moistening effect and the minimum toxicity of this solution makes it a good choice for wound care management. This antibiotic technology offers a broad paradigm for prevention and treatment of acute and chronic wounds.

Gregio et al (2006)[31] checked the antimicrobial activity of *Zingiberofficinale* on the pathogens found in the oral cavity which included the *Candida*

albicans also. Oral flora is one of the places where it has bigger variety of micro organisms and becomes pathogenic due to its unstability. The extract showed excellent antibacterial and antifungal effects and was hence concluded as a promising contribute to the treatment of illnesses caused by these micro organisms in the oral cavity.

Hui-Jon Choi et al (2006)[32]studied the effect of chlorhexidine on wound healing and it has been concluded that chlorhexidine affects the formation of bone nodules by human fibroblasts and hence it may be prudent to avoid contact with chlorhexidine until wound healing is well advanced. The reduction in concentration of chlorhexidine had no effects on cellular proliferation.

Payal Patel et al (2006)[33] investigated the cytotoxicity of chorhexidine mouthwash using a cell culture model by employing an assay to record cell activity. The cells were exposed to chlorhexidine mouthwash for 5 minutes to 4 hours. Undiluted chlorhexidine mouthwash showed total cytotoxicity. Progressive dilution of chlorhexidine mouthwash was associated with elevated cell survival. The study showed that higher concentrations and more the time of exposure showed increased cytotoxicity.

Nagham H. kassab et al (2007)[34] in their study evaluated the effectiveness of different extracts of Curcumine on *Candida* biofilm removal from acrylic denture base resin material. Curcumine belonged to the ginger family and their effects were seen to vary with the type of extract. Study results showed maximum effectiveness for ethanolic extract of curcumine and was suggested as a new denture cleansing agent in Prosthetic dentistry.

Sabrina Pavan et al (2007)[35] evaluated the effectiveness of disinfection treatments with chemical solutions-2% glutaraldehyde,5% sodium hypochlorite, 5% chlorhexidine and microwave energy on the hardness of 4 long term soft denture liners. The hardness values of the soft denture liners were measured before and after disinfection. It was seen that the number of disinfections had no effect on the hardness values for all the materials studied and disinfection techniques. It was concluded that the 2 disinfection cycles did not change the hardness values for all the materials. Here glutaraldehyde demonstrated highest hardness values for Molloplast B whereas eversoft did not present any difference with different disinfection treatments.

Tatiana Pereira et al (2008)[36]described several key factors controlling the adhesion of *Candida* species which are relevant to denture associated

stomatitis. The surface properties have shown to have some role but studies on several other factors like use of denture liners, salivary properties, yeast-bacterial interactions have shown contradictory findings. The contribution of saliva is also unclear due to its variations of collections and handling. The experimental procedures should be standardized to bridge the gap between the laboratory studies and clinical findings.

Bilge T. Bal et al (2008)[37] compared the adhesion of oral micro organisms to different types of soft liner and acrylic resin surfaces. It was seen that higher numbers of oral bacteria and *Candida* were shown to adhere to soft lining materials than to acrylic resin. It was also seen that the microbial coverage increased continuosly with time. It was hence concluded that temporary soft lining materials are not resistant to adhesion and possible surface damage caused by oral bacteria, and therefore their use is to be limited to short term periods.

A. DilekNalbant et al (2008)[38] in this study focuses on the effectiveness of Klorhex and Fittydent ,which are used as cleaning agents on the adhesion of *Candida* on the surfaces of acrylic denture and palatal mucosa. Also the adherence capacity of these yeasts to acrylic strips was evaluated. The results showed that the application of these cleaning agents

modulates the Candidal adherence to denture acrylic and that they do affect the colonization rate of *Candida albicans* on the surface of dentures and palatal mucosa.

Ralf Beurgers et al (2008)[39] compared the efficacy of 10 denture disinfection methods in reducing *Candida albicans* colonization on soft lining materials. They concluded from the results obtained that only soaking in sodium hypochlorite, microwave irradiation in water and application of effervescent cleansing tabs proved to be effective against *Candida albicans* colonization on soft lining materials.

Francine Cristina da silva et al (2008)[40] compared the effectiveness of various disinfectant solutions in the disinfection of acrylic resin specimens contaminated in vitro by *Candida albicans* and other few micro organisms. The suface roughness influence was also monitored separately. The results showed and it was concluded that1% sodium hypochlorite, 2% glutaraldehyde ,2% chlorhexidine, 100% vinegar , 3.8% sodium perborate are all valid alternatives for the disinfection of acrylic resins.

Maria et al (2009)[41] assessed the efficacy of denture cleansers on *Candida albicans* and *Candida glabrata* adherence on denture liners. Three denture liners were treated with sodium hypochlorite (NaOCl) disinfectant at

various time exposures and was found to be very effective to reduce the candidal adherence on all materials tested.

Zahra Atai et al (2009)[42] in their study attempted to find an alternative to antifungal drug Nystatin to overcome the problems caused by its usage. Laboratory investigation regarding the antifungal effect of Ginger (*Zingiberofficinale*) was done on *Candida albicans*. Ethanolic extract of ginger was prepared and the antifungal effects verified. It was concluded and recommended from this study that the ethanolic extract of ginger was effective on *Candida albicans* and it showed a promising treatment for oral candidasis.

Lin Biao-sheng et al (2009)[43] tested the ethanolic extracts of ginger, garlic, hot pepper for their antimicrobial effects against various micro organisms including the *Candida albicans*. The results showed that all the extracts have effective antimicrobial activities against bacteria, moulds and yeast. They showed a broad spectrum of antimicrobial activities. This study also proved that the effectiveness improved with increments in concentration. The minimum inhibitory concentrations are varied with different micro organisms and extracts.

Melvin Joe et al (2009)[44] verified the effectiveness of *Zingiberofficinale*(ginger) and few other extracts over few micro organisms including *Candida albicans.* The antimicrobial activity of ginger was attributed to antimicrobial substances such as Zingiberol, Zingiberine and bisabolene. It also contains pungent vanillyl ketones including gingerolandparadole.

Marcelo Coelho Goiato et al (2010)[45] studied the colour change of soft liners after thermocycling and storage in coffee and coke was observed. It was seen that coke did not influence the colour stability whereas coffee was seen to generate a statistically significant colour alteration. Also, the silicone liners showed better colour stability following thermocycling and storage independent of the solution.

H Tan et al (2000)[46] in their study compared the colour ,texture and Shore a hardness of a resilient silicone denture liner with as – polymerised, roughened or pumice surfaces after treatment with perborate, persulfate or hypochlorite containing denture cleansers. It was seen that the roughened surfaces exhibited significant colour loss with some perborate containing cleansers. No difference in surface texture was observed upon the cleanser treatment. Colour loss was observed due to the leaching out of components from the liner.

MATERIALS AND METHODS

The disinfectants used in this study were (Figure 1):

1. Superoxidised solution (Figure 1a),
2. Extract of *Zingiberofficinale* (Ginger) (Figure 1b),
3. Extract of *Zingiberwightianum* (Hill Ginger) (Figure 1c),

   The effectiveness of the disinfectants were assessed for various times of exposures.

The Superoxidised solution used was a commercially available product [Oxum, Alkem laboratories ltd, Batch No. 91400008]. *Zingiberofficinale* (Ginger) extract and *Zingiberwightianum*(Hill Ginger) extract were prepared in the laboratory. Distilled water was used as a control solution against the experimental solution of Superoxidised water whereas alcohol (methanol) was used as control solution against the experimental extract solutions of *Zingiberofficinale* (Ginger) and *Zingiberwightianum*(Hill Ginger).

A total of 90 specimens of the silicone based soft liner was prepared and smeared with *Candida albicans* in this study. The efficacy of the 3 disinfectants was assessed at various time of exposures- 1 minute, 5 minutes and 30 minutes. 6 samples were used for each of the treatments and controls under each time of exposure.

Methodology

The rhizomes were collected from Kottayam district of Kerala. They were washed, sliced, dried in the shade and powdered. Soxhellet apparatus [ROTEK, Sl No. 0531, B & C Industries, Vengola, Kerala] (Figure -3) was used for the extraction of the rhizome extracts. A solvent was required for the extraction of the extract from the powdered rhizome and hence alcohol, which is a commonly used solvent for plant extracts was used. 12 grams of the powdered rhizome was measured and 170 ml of alcohol was taken. It was subjected to Soxhellet extraction with methanol until a clear extract was obtained. After the extraction process, the powder was weighed again and the concentration of the extract was determined based on the difference in the amount of powder that was taken initially and the amount of powder remaining after the extraction process. *Candida albicans* species isolated from the oral cavity was used for the study which was confirmed by germ tube formation.

Sabourauds dextrose broth (containing mycological peptone, anhydrous dextrose, distilled water) was prepared, autoclaved and dispensed into sterile test tubes (2ml). Cultured *Candida albicans* was inoculated into these test tubes using inoculation loops. After inoculation, it was kept for incubation for

1 hour in an incubator [BESTON, Cat No. B 29701, Beston industries, Cochin, Kerala](Figure -4). The solution becomes turbid due to the growth of *Candida* over the period of incubation. The silicone based soft liner (Mucopren soft, KettenbachGmbh& Co. KG, Germany, 20907/1708) (Figure -2) specimens of dimension 2cm long x 1cm wide x 2mm thick was prepared in stainless steel moulds. The specimens were placed into these test tubes containing the *Candida* inoculated broth and kept for a 2 hour incubation. After 2 hours the specimens were carefully taken out and put into the disinfectant solutions and the controls for the respective time durations of 1 minute, 5 minutes, 30 minutes. The liner specimens were then placed into test tubes containing distilled water (2ml). This was then subjected to vortexing in a test tube shaker [REMI Cyclomixer, CM 101, India] (Figure -5) for a time period of 5 minutes. 0.1 micro ml was pipetted out carefully from the test tube using a micro pipette and this drop was placed onto the plates containing Sabourauds dextrose agar medium. The drop was evenly spread throughout the plate using a glass L-rod. These plates were then incubated at 37^0C for 24 hours.

Distilled water was used as a control solution against the experimental solution of Super oxidized water whereas alcohol (methanol) was used as the

control solution against the experimental extract solutions of *Zingiberofficinale* (Ginger) and *Zingiberwightianum* (Hill Ginger).

Observations and recordings were noted after the time period of incubation.

In addition, zones of inhibition were also analysed to check the effectiveness of the Zingiber family extracts against the growth of *Candida albicans*. Pertiplates containing Sabourauds dextrose agar medium were taken. *Candida albicans* was streaked onto the plates using a sterile cotton bud. A well was created at the centre of the plate and the extract drops were placed into the well. The plates were kept for incubation at 37^0C for 24 hours, the zones of inhibition were measured and the values noted.

Standard microbiological methods were used for culture of *Candida albicans*. Protective surgical masks, sterile gloves were worn to ensure personnel protection from the pathological organism.

a **b** **c**

Figure 1
Materials used in the study

Figure 2
Silicone based soft liner

Figure 3
Soxhellet apparatus

Figure 4
Incubator

Figure 5
Test tube shaker

RESULTS AND OBSERVATIONS

The study was completed and the results were tabulated as shown in Tables I to VI.

Tables I-V showed the basic data for the colony forming units of *Candida albicans* after exposure to the various disinfectants at different time intervals. Among the experimental treatments, Ginger (*Zingiberofficinale*) showed the maximum effectiveness. There is complete disinfection seen at exposures of 5 minutes and 30 minutes.

Hill ginger (*Zingiberwightianum*) was seen to be the next most effective treatment solution. It showed complete disinfection at 30 minutes of exposure.

Superoxidised water (Oxum) did not show complete disinfection of the *Candida* infected soft liners. But they were seen to exhibit maximum effectiveness in the shortest period of time. The colony count was seen to reduce by 98.98% in 1 minute of exposure and their effectiveness subsequently decreased on prolonged exposures.

Table VI shows the basic data of the zone of inhibition measurements and their mean values.

Zingiberofficinale (Ginger) showed a higher value for zone of inhibition than *Zingiberwightianum*(Hill Ginger) thus exhibiting more effectiveness.

Table VII shows the mean values of the colony forming units of *Candida albicans* after exposure to the various disinfectants at different time intervals.

Table VIII shows the percentage effectiveness of the disinfecting solutions.

Tables IX shows One way ANOVA statistical analysis done for the comparison of disinfectants after exposure for each time interval. The p value obtained was < .01 and hence it showed significant difference among the disinfectant solutions.

Table X shows One way ANOVA statistical analysis done for the comparison of the efficacy of various disinfectants in different time intervals in the study.

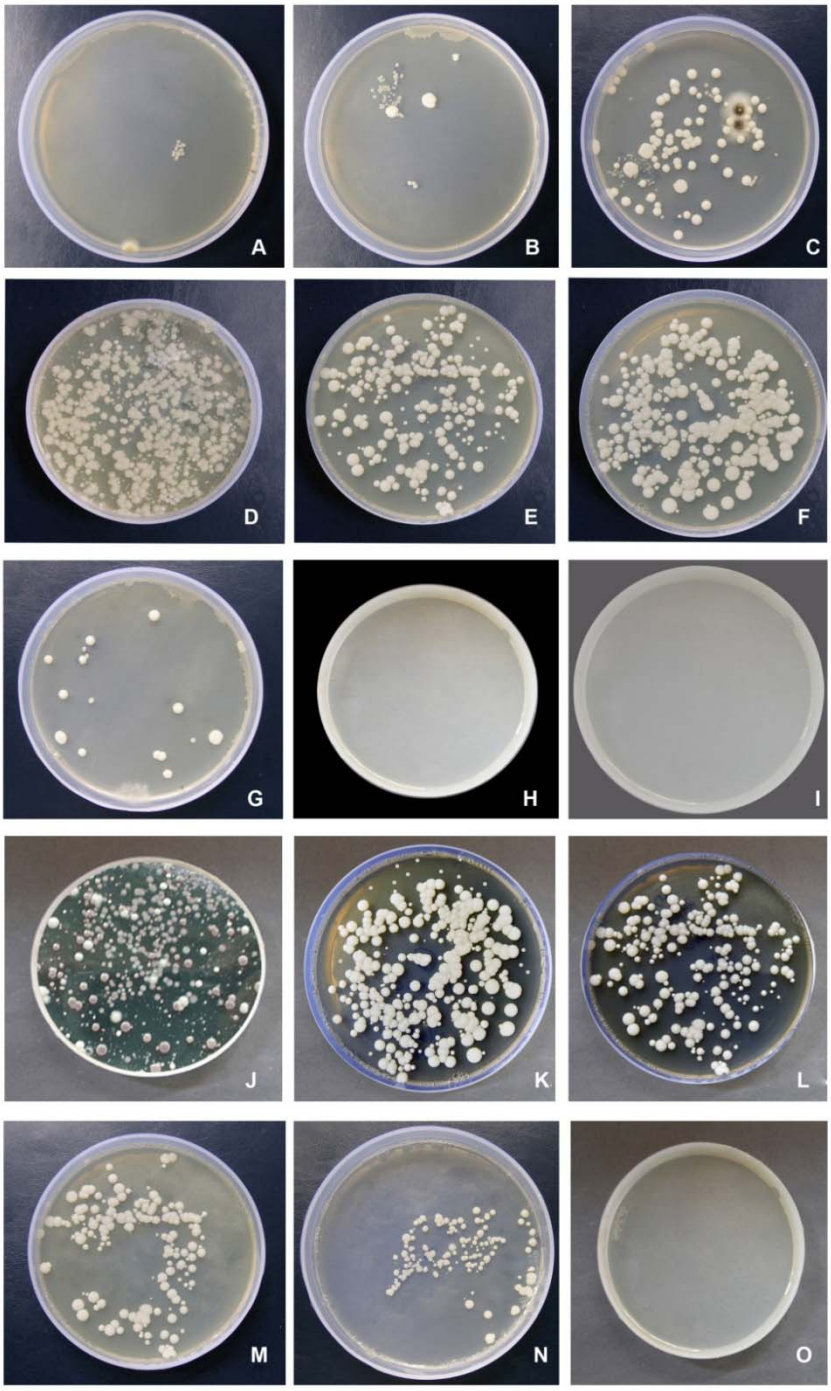
The p value obtained was <.01 and hence it showed significant difference among the various time of exposures.

Table XI shows the statistical analysis for the comparison of the zone of inhibition values of the Zingiberacea (Ginger) family. Here also the p value obtained was <.01 and hence it showed significant difference among the effectiveness of the Zingiberacea (Ginger) family extracts

A graph (Figure 8) was plotted for the number of *Candida* colony forming units against the various time of exposures. Ginger and Hill Ginger showed a steady decrease in the number of colony forming units of *Candida albicans* as the time of exposure increased whereas Superoxidised water showed a steep decrease initially and then a gradual increase in the count of *Candida albicans* colony forming units.

Figure 6

CFU of *Candida albicans* in soft liners after exposure to disinfectants and controls at different time intervals



A- Superoxidised water (1 minute)

B- Superoxidised water (5 minutes)

C- Superoxidised water (30 minutes)

D- Distilled water control (1 minute)

E- Distilled water control (5 minutes)

F- Distilled water control (30 minutes)

G- *Zingiberofficinale* (1 minute)

H- *Zingiberofficinale* (5 minutes)

I- *Zingiberofficinale* (30 minutes)

J- Alcohol control (1 minute)

K- Alcohol control (5 minutes)

L- Alcohol control (30 minutes)

M-*Zingiberwightianum* (1 minute)

N- *Zingiberwightianum* (5 minutes)

O- *Zingiberwightianum* (30 minutes)

Zones Of Inhibition

Figure 7

Zingiberofficinale ***Zingiberwightianum***

Table I

Basic data of CFU of *Candida albicans* in soft liner after exposure to Superoxidised solution at different time intervals

Disinfecting solution	1 minute (CFU)	5 minutes (CFU)	30 minutes (CFU)
Superoxidised water	8×10^6	67×10^6	162×10^6
	2×10^6	80×10^6	122×10^6
	8×10^6	98×10^6	165×10^6
	2×10^6	55×10^6	112×10^6
	3×10^6	78×10^6	148×10^6
	7×10^6	82×10^6	132×10^6

Table II

Basic data of CFU of *Candida albicans* in soft liner after exposure to Distilled water (control) at different time intervals

Disinfecting Solution	1 minutes (CFU)	5 minute (CFU)	30 minutes (CFU)
Distilled water (control)	458×10^6	226×10^6	224×10^6
	529×10^6	152×10^6	186×10^6
	451×10^6	150×10^6	198×10^6
	534×10^6	200×10^6	190×10^6
	442×10^6	182×10^6	220×10^6
	544×10^6	224×10^6	204×10^6

Table III

Basic data of CFU of *Candida albicans* in soft liner after exposure to *Zingiber officinale* (Ginger) extract at different time intervals

Disinfecting Solution	1 minutes	5 minute	30 minutes
Zingiber officinale **(Ginger)**	**15×10^6** **20×10^6** **22×10^6** **12×10^6** **10×10^6** **15×10^6**	**nil**	**nil**

Table IV

Basic data of CFU of *Candida albicans* in soft liner after exposure to *Zingiber wightianum* (Hill Ginger) extract at different time intervals

Disinfecting Solution	1 minutes	5 minute	30 minutes
Zingiber officinale (Ginger)	**200×10^6** **190×10^6** **150×10^6** **185×10^6** **193×10^6** **195×10^6**	**155×10^6** **144×10^6** **100×10^6** **94×10^6** **117×10^6** **125×10^6**	**nil**

Table V

Basic data of CFU of *Candida albicans* in soft liner after exposure to Alcohol (control) at different time intervals

Disinfecting Solutions	1 minute	5 minutes	30 minutes
Alcohol (control)	241×10^6	225×10^6	190×10^6
	263×10^6	218×10^6	184×10^6
	235×10^6	223×10^6	175×10^6
	266×10^6	232×10^6	192×10^6
	262×10^6	223×10^6	188×10^6
	248×10^6	220×10^6	190×10^6

Table VI

Basic data and Mean values of Zone Of Inhibition Measurements

Sl No.	Disinfectant Solution	Zone of Inhibition (In mm)	Mean Value of Zone of Inhibition (In mm)
1	Ginger (*Zingiber officinale*)	66 68 70 65 66 60	65.83
2	Hill Ginger (*Zingiber wightianum*)	52 54 52 50 53 55	52.6

Table VII

Mean value of CFU of *Candida albicans* in denture soft liner after exposure to disinfecting agents at different time intervals

Sl. No	**Disinfecting solution**	**Total count of *Candida albicans* before disinfection**	**1 minute**	**5 minutes**	**30 minutes**
1	Superoxidised water	493×10^6	5×10^6	76.66×10^6	140.16×10^6
2	Distilled Water [***Control for 1***]	493×10^6	493×10^6	189×10^6	203.66×10^6
3	*Zingiber officinale* (Ginger)	493×10^6	15.66×10^6	nil	nil
4	*Zingiber wightianum* (Hill Ginger)	493×10^6	185.5×10^6	122.5×10^6	nil
5	Alcohol [***Control for 3&4***]	493×10^6	252.5×10^6	223.5×10^6	186.5×10^6

TABLE VIII

Percentage effectiveness of the disinfecting solutions

Sl. No.	**Disinfecting solution**	**1 minute**	**5 minute**	**30 minute**
1	Super oxidized water	98.98%	84.44%	71.56%
2	Distilled water (control)	No effect	61.65%	58.68%
3	*Zingiber officinale* (Ginger)	96.81%	100%	100%
4	*Zingiber wightianum* (Hill Ginger)	62.37%	75.14%	100%
5	Alcohol (control)	48.87%	54.66%	62.16%

TABLE - IX

One Way ANOVA test to compare the efficacy of disinfectants after exposure for each time interval

Disinfectant	**Mean value**	**F value**	**p value**	**CD(5%)**
	After 1 minute of exposure			
1.Superoxidised water	$5x\ 10^6$			
2.Distilled water	$493\ x10^6$			
3.*Zingiber officinale*	$15.66\ x\ 10^6$	435.85	< 0.01	$27.94\ x\ 10^6$
4.*Zingiber wightianum*	$185.5\ x10^6$			
5.Alcohol	$252.5\ x\ 10^6$			
	After 5 minutes of exposure			
1.Superoxidised water	$76.67\ x10^6$			
2.Distilled water	$189\ x\ 10^6$			
3.*Zingiber officinale*	0	122.61	< 0.01	$23.32\ x\ 10^6$
4.*Zingiber wightianum*	$122.5\ x\ 10^6$			
5.Alcohol	$223.5\ x\ 10^6$			
	After 30 minutes of exposure			
1.Superoxidised water	$140.17\ x\ 10^6$			
2.Distilled water	$203.67\ x\ 10^6$			
3.*Zingiber officinale*	0	119.36	< 0.01	$18.65\ x\ 10^6$
4.*Zingiber wightianum*	0			
5.Alcohol	$186.5\ x\ 10^6$			

TABLE - X

One way ANOVA test to compare the efficacy of the various disinfectants in different time intervals in the study

Time	Mean value of CFU of *Candida albicans*	F value	p value	CD(5%)
	Super oxidized water			
1 minute	5×10^6	119.36	< 0.01	18.65×10^6
5 minutes	76.7×10^6			
30 minutes	140.16×10^6			
	Distilled water (control)			
1 minute	493×10^6	146.68	< 0.01	42.64×10^6
5 minutes	189×10^6			
30 minutes	203.67×10^6			
	Zingiber officinale			
1 minute	15.67×10^6	69.91	< 0.01	69.91×10^6
5 minutes	0			
30 minutes	0			
	Zingiber wightianum			
1 minute	185.5×10^6			
5 minutes	122.5×10^6	177.52	< 0.01	21.33×10^6
30 minutes	0			
	Alcohol (control)			
1 minute	252.5×10^6			
5 minutes	223.5×10^6	85.309	< 0.01	10.79×10^6
30 minutes	186.5×10^6			

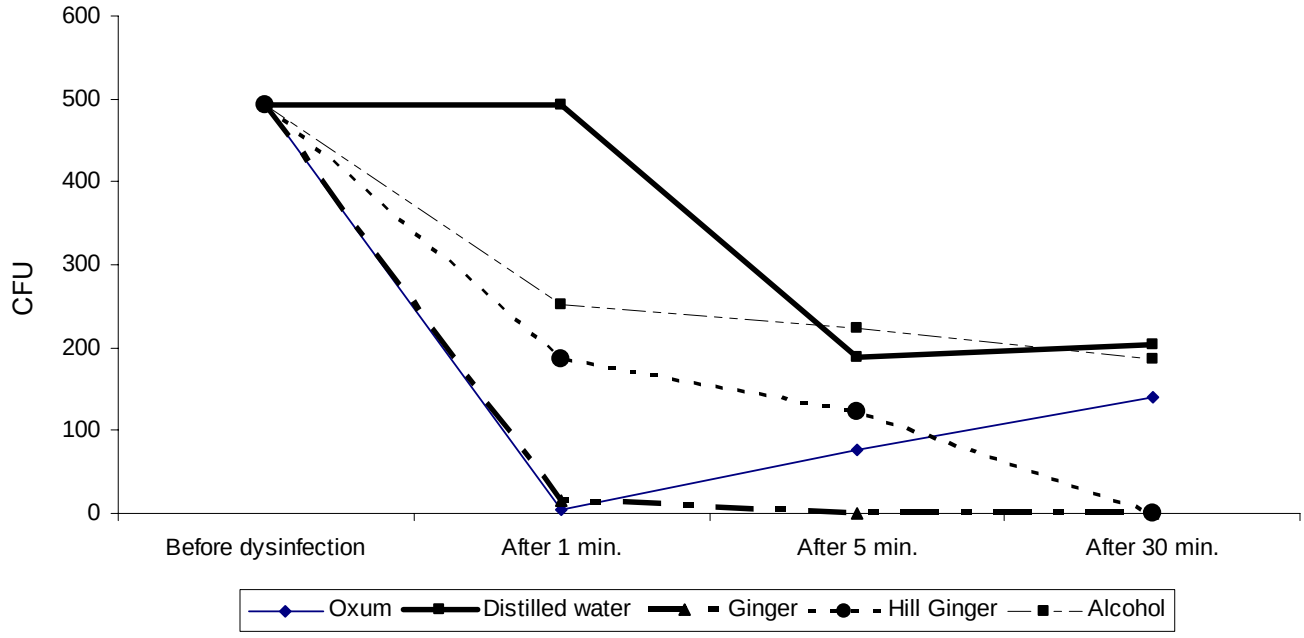
Table XI

Zone of Inhibition-Statistical analysis

Sl No.	Disinfectant	Mean	F Value	p Value
1	Ginger (*Zingiber officinale*)	65.83	72.07**	< .01
2	Hill ginger (*Zingiber wightianum*)	52.67		

Figure 8

Effect of various disinfectants on *Candida albicans* on denture soft liners



DISCUSSION

Denture soft liners provide comfort in a huge manner to the denture wearing patient as it acts as a cushion between the denture base and the oral mucosa. The main problem of concern is the microbial colonization of the denture soft liner over the time period of usage. Colonisation by the yeast, *Candida albicans* is of a major concern as it leads to a pathological state of the oral cavity-Oral Candidasis. Even though many other micro organism including other species of *Candida* are found to be colonizing in the soft liner, *Candida albicans* is found to be the main causative organism for the same[2]. This can further lead to Denture stomatitis and also angular cheilitis accompanied by it.

The daily cleansing methods used to cleanse a denture , being used for the soft liner does not help in the prevention of the microbial colonization . Extra cleansing treatment regimens need to be followed by the patient to disinfect the soft liners and prevent a state of pathology.

Many commonly used chemical disinfectants are available and used for the same on denture soft liners. All these chemicals have shown cytotoxic effects on the human tissues[2,8,33,34]. Hence this study has shifted towards testing more nature based disinfecting products and their effectiveness on disinfection of *Candidaalbicans* from denture soft liners.

Super oxidized solution is basically water with many oxidising agents incorporated in it. The cleansing action takes place due to the release of nascent oxygen . In this study the effectiveness of superoxidised solution on *Candida albicans* in soft liners has been checked during varying time of exposures. The results show that the efficacy of the superoxidised solution on *Candida albicans* was decreasing with increased time of exposure. The reason could be explained as once exposed, the stability of the superoxidised solution deteriorates. The oxidizing agents present in the solution are slowly released and lost and hence the effectiveness of the solution decreases with increasing time. In this study, maximum effectiveness for superoxidised solution was seen at the time of exposure of 1 minute. Greater number of colony forming units of*Candida albicans* was seen at 5 minutes and even greater number was seen at 30 minutes after exposure.

Various studies showed a highly significant effect of superoxidised solution on *Candida albicans*. The maximum effectiveness of the solution was seen at less than 2 minutes and at higher time of exposures, the effectiveness was noted to be decreasing.[12,13,26,28] Distilled water was used as the control here. The major advantage of superoxidised solution was the maximum disinfection at the shortest time. Even though it has been reported that it shows complete disinfection of *Candida* species, in this study 100%

effectiveness is not seen on the disinfection of *Candida albicans* adhered to soft liners. Superoxidised solution is basically used for the treatment of wound infections (eg: diabetic foot) and hence it shows a wide range of antimicrobial activity[30]. Its effectiveness of disinfecting *Candida albicans* infected soft liners have been evaluated in this study and was found that complete disinfection of the soft liner does not take place. Some amount of *Candida* colonies remain on the liner as it shows only 98.98% effectiveness in its most effective time period of action.

Herbal formulations have always been preferred by patients as medications since cytotoxic effects are minimized when compared to the chemicals commercially available. Many studies have shown Ginger family (Zingiberacea) to be having a high antifungal potential[23,43-45]. In this study the antifungal efficacy of 2 rhizomes belonging to the Zingiberacea family- *Zingiberofficinale* (Ginger) and *Zingiberwightianum*(Hill ginger) have been analysed. Significant anti candidal effect was shown by Ginger extract on oral species of *Candida albicans*. No studies have been published earlier regarding the antifungal potential of *Zingiberwightianum*(Hill Ginger).

Methanolic extracts of the rhizomes prepared in the laboratory using the Soxhellet apparatus was used for the study and alcohol as control solution.

This study focused on the effectiveness of Ginger extract in disinfection of *Candida albicans* infected soft liners. *Zingiberofficinale* (Ginger) extract has shown a higher disinfection potential in a shorter period of time than the *Zingiberwightianum*(hill ginger)extract.

Zone of inhibitions were observed for the two variants of Ginger after a 24 hour period of incubation and their measurements valued. Statistically significant differences were found to be there between the zone measurements of the two. *Zingiberofficinale* showed a highly significant effect against the growth of *Candida albicans* compared to the *Zingiberwightianum*. Testing for zones of inhibition for super oxidized solution is of no significance as the zone measurements can be recorded only after a 24 hour time period whereas the superoxidised solution loses its effectiveness after a very short time period.

The high anti-candidal potential would be due to its antimicrobial components such as Zingiberol, Zingiberene and Bisabolene. It also contains pungent vanillyl ketone including gingerol and paradole etc. Gingerole is a mixture of crystal gingerone and it is the major cause of acidity of ginger[32,44]. From this study results it suggests the ginger components as promising

candidates for the development of disinfecting solutions for the disinfection of *Candida albicans* infected soft liners.

Superoxidising solution being highly effective in the shortest time of exposures, can be used in the form of sprays for the disinfection of impressions, denture bases, dentures lined with soft liners. The patient can use it for a quick disinfection after a meal as it does not require long time of exposures. Ginger showing such promising results, can be incorporated into disinfecting solutions giving a herbal based product and the patient can be advised to use it for an overnight soak of the denture for effective disinfection of *Candida* from the soft liner.

CONCLUSION

This study was conducted to test the efficacy of 3 new disinfectants on its ability to remove *Candida albicans* adhered to silicone based denture soft liners.

The conclusions were:

i. *Zingiberofficinale* showed the maximum effectiveness of all the three disinfectants
ii. The effectiveness of superoxidised water was seen to be reducing with increased time of exposure. Hence its maximum effect was seen in the least time exposure
iii. The effectiveness of the herbal formulations (*Zingiberofficinale*, *Zingiberwightianum*) was seen to increase with the increased time of exposure.

BIBLIOGRAPHY

1. **Mahmoud Khamis, Zakia Mohamed**. Influence of Tissue conditioning materials on the oral bacteriologic status of Complete denture wearers. J Prosthet Dent Aug 1980; Vol 44 No.2.
2. **LasseAnsgar and Erik Holst**. Desquamative mucosal reactions due to Chlorhexidinegluconate: Report of 3 cases. International Journal of Oral Surgery Vol 11, Issue 6, Dec 1982, 380-382.
3. **P.S Wright, P. Clark and J.M Hardie.** Clinical Science The Prevalence and Significance of Yeasts in Persons wearing Complete Dentures with Soft- Lining Materials. J Dent Res 1985;64:122.
4. **Jane McCourtie, T.W MacFarlane and L.P Samaranayake.** Effect of ChlorhexidineGluconate on the adherence of *Candida* species to denture acrylic. J Med Microbiol 20 (1985), 97-104.
5. **Sharon D. Hill, Charles W. Berry .** Comparison of antimicrobial and cytotoxic effects of Glutaraldehyde and Formocresol.Oral Surgery, Oral medicine, Oral pathology Vol 71, Issue 1, January 1991, Pages 89 95.
6. **Crispian Scully**Candidasis, Mucosal

7. **P.S Wright** . Observations on the long term use of a soft lining material for mandibular complete dentures. J Prosthet Dent 1994;72 ;385-92.
8. **Babich H, Wurzburger BJ, Rubin YL, Sinensky MC, BlauL .** An in vitro study on the cytotoxicity of Chlorhexidinegluconate to human gingival cells. Cell BiolToxicol 1995 Apr: 11(2): 79-88.
9. **M.G.J Waters, D.W Williams, R.G Jagger, M.A O Lewis.** Adherence of *Candida albicans* to experimental denture soft lining materials. J Prosthet Dent 1997; 77:306-12.
10. **Tae YounChoi, Won Bae Kim.** Bactericidal effect of a disinfectant a Superoxidised water, Medilox .Korean Journal of Nosocomial infections Vol3, No.1, May 1998.
11. **Pizzo G, Giulana G.** Antifungal activity of Chlorhexidine containing mouthrinses- An invitro study. Minerva Stomatol 1998 Dec;47 (12): 665-7
12. **Selkon JB, Babb JR, Morris R.** Evaluation of the antimicrobial activity of a newsuperoxidised water, Sterilox, for the disinfection of endoscopes. J Hosp Infect 1999 Jan: 41(1): 59-70.
13. **Shetty N, Srinivasan S, Holton J, Ridgway GL.** Evaluation of Microbicidal activity of a new disinfectant: Sterilox 2500 against

Clostridium difficile spores, Helicobacter pylori, Vancomycin resistant Enterococcus species, *Candida albicans* and several Mycobacterium species. J Hosp Infect 1999 Feb;41(2): 101-5.

14. **Susan L. Zunt** Oral Candidasis: Diagnosis and treatment. Journal of Practical hygiene September/October 2000.

15. **Middleton A.M, Chadwick MV, Sanderson JL, Gaya H**

Comparison of a solution of superoxidised water with Glutaraldehyde for the disinfection of bronchoscopes, contaminated. J Hosp Infect 2000 Aug; 45(4): 278-82.

16. **KishiiJiro, Yamaguchi Mutsuo, Sakai Makoto, Omiya takashi, IshiguchiTakehiro, NagasawaTooru.**Effect of electrolysed water on Resilient denture liners. Journal of the Japan Prosthodontic society Vol 44, no.5, 643-650 (2000).

17. **Richard D Cannon, LaJean Chaffin.** Colonisation is a crucial factor in Oral Candidiasis. Journal of Dental Education Aug 2001, Vol 65, No.8.

18. **William A. Rutala and David J. Weber.** New Disinfection and Steriliation methods. Emerging Infectious diseases Vol.7, No.2, March-April.

19. **Yuki Nagamatsu, Kiyoshi Tajima, Hiroshi Kakigawa and Yoshio Kozono.** Application of Electrolysed acid water to sterilization of Denture base. Dental materials Journal Vol 20, No.2 (2001) 148-155.

20. **AAkpan, R. Morgan.** Oral Candidiasis March 2002

21. **Mary E Brosky, Igor J. Pesun, Brad Morrison, James S. Hodges, Juey H Lai and William Liljemark.** Clinical Evaluation of resilient Denture liners. Part 2: *Candida* count and Speciation, Journal Of Prosthodont 2003;12:162-167.

22. **M Urata, H Isomoto, K Murase, A Wada, K Yanagihara, Y Hirakata, F Takeshima, k Omagari, Y Mizuta, I Murata, S Kohno.** Comparison of the microbicidal activities of Superoxidised and Ozonated water in the disinfection of endoscopes. Journal of International medical research 2003;31:299-306.

23. **C Ficker, M.L Smith, K Akpagana, M. Gbeassor, J Zhang, T Durst, R Assabgui, J.T Amason.** Bio assay guided isolation and identification of antifungal compounds from Ginger. Phytotherapy research Sep 2003, Vol 17, issue 8, 897-902.

24. **Renata M C Rodrigues Garcia, Blanca T L Leon, Viviane B M Oliveria and Altair A Del BelCury.** Effect of a denture cleanser on

weight, surface roughness, and tensile bond strength of two resilient denture liners. Dent Mater J.

25. **Bulad K, Taylor RL, Verran J, McCord JF.**Colonisation and penetration of denture soft lining materials by *Candida albicans*. Dent mater 2004, Feb 20 (2): 167-75.

26. **Landa Solis C, Gonsalez-Espinosa D, Guzman-Soriano B, Snyder M, Reyes-teran G, Torres k, Gutierrez AA .** Microcyn: a novelsuperoxidised water with neutral pH and disinfectant activity. J Hospinfect. 2005 Dec;61(4):291-9 Epub 2005.

27. **McCullough MJ, Savage NW** . Oral Candidosis and the therapeutic use of antifungal agents in dentistry. Aust Dent J. 2005 Dec;50(4 Suppl 2): S 36-9.

28. **DouglasGBenting, Igor J. Pesun and James Hodges.** Compliance of Resilient denture liners Immersed in Effervescent denture cleansers. Journal of Prosthodontics Vol 14, No.3 September 2005;175-183.

29. **K. Rideout, K Teschke, H. Dimich-Ward, S M. Kennedy.** Considering risks to healthcare workers from Glutaraldehyde alternatives in high level disinfection . Journal of Hospital infection (2005) 59,4-11.

30. **Andres A. Gutierrez** . The Science behind Stable, Super-oxidised water Supplement to Jan 2006 Wounds.

31. **Gregio, Fortes, Rosa, Simeoni.** Antimicrobial activity from ZingiberOfficinale on oral cavity pathogens . Biology studies Vol 28. No.62, March 2006.

32. **Hui-Jun Choi, Suk Ji ,Joong Ki Kook, Hyun Seon Jang, Joo-Cheol park.** The effect of Chlorhexidine on the formation of bone nodules by periodontal ligament cells in vitro. Korean dental J Vol 36,No.2, 2006.

33. **Payal Patel, Mark Ide, Paula Coward, Lucy Di Silvo.** The effect of a commercially available mouthwash product on human osteoblast cells.

34. **Nagham H. Kasab, Eman A. Mustafa, Maha T. Al Saffar.** The ability of different Curcumine solutions on reducing *Candida albicans* biofilm activity on acrylic resin denture base material . Al Rafidain Dent J 2007;7(1):32-37.

35. **Sabrina Pavan, Joao NeudenirArioliFilho, Pulo Henrique Dos Santos, Sergio SualdiniNogueira, Andre UlissesDantas Batista.** The effect of disinfection treatments on the hardness of soft denture liner materials. Journal of Prosthodontics March/April 2007 Vol 16, Issue 2, 101-106.

36. **TatianaPereira-Cenci, Del BelCury, Crielaard, Ten Cate** Development of *Candida* associated denture stomatitis: New insights. J appl Oral Sci, 2008;16(2):86-94.

37. **Bilge T.Baletal** A pilot study to evaluate the adherence of oral micro organisms on temporary soft lining materials. Journal of Oral science Vol 50, No.1, 1-8,2008.

38. **A. DilekNalbant, AyseKalkanci, BanuFliz, SemraKustimur.** The effectiveness of different cleaning agents against the colonisation of Canidida species and the invitro detection of the adherence of these yeast cells to denture acrylic surfaces. Yonsei Med J.2008 August 30.

39. **Ralf Beurgers, Martin Rosenstritt, Wulf Schneider-Brachert, Gerhard Handel, Sebastian Hahnel** Efficacy of denture disinfection methods in controlling Candida albicans colonization in vitro. ActaOdontologicaScandinavicaVol 66 Issue 3 2008, 174-180.

40. **Francine Cristina da silva, Kimpara, Maria Nadir, Ivan Balducci, Antonio Olavo and Cristiane** Effectiveness of six different disinfectants on removing five microbial species and effects on the topographic characteristics of Acrylic resin. Journal of Prosthodontics Dec 2008,Vol 17,Issue 8, 627-633.

41. **Maria Ferreira, Tatiana Pereira-Cenci, LuciolaVascocelos, Renata Cunha-Garcia and Altair Del BelCury.** Efficacy of denture cleansers on denture liners contaminated with Candida species. J of Clinical oral investigations, Vol 13, No.2, June 2009.

42. **Zahra Atai, ManijehAtapour and Maryam Mahseni .**Inhibitory effect of Ginger extract on *Candida albicans* . American Journal of applied sciences 6(6): 1067-1069,2009.

43. **Lin Biao-Sheng,Zhang Ting-ting,Shenshao-xin** Research on antimicrobial activities of ethanol extracts from Ginger, garlic and Hot pepper. Journal of Longvan University 2009.

44. **M. Melvin Joe, Jayachitra and M. Vijayapriya.** Antmicrobial activity f some common spices against certain human pathogens. Journal of medicinal plants research Vol 3(11) 1134-1136, Dec 2009.

45. **Marcelo CoehloGoiato, Bruna Carolina, Amalia Moreno, Daniela Michelin.** Colour changes of soft denture liners after storage in coffee and coke. Gerodontology 2010.

46. **H. Tan, A Woo,S Kim, M Lamoureux, M Grace.**Effect of denture cleansers, surface finish, temperature on Molloplast B resilient liner color, hardness and texture. Dent Mater J